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Effect of time before storage and storage temperature on survival of *Salmonella* inoculated on fresh-cut melons ☆

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Abstract

The effects of a waiting period at room temperature (~22 °C) before refrigerating fresh-cut watermelon, cantaloupe and honeydew pieces contaminated with *Salmonella* on survival of the inoculated pathogen were investigated. Whole cantaloupes, honeydew melons and watermelons were washed with water, and fresh-cut pieces from individual melons were prepared and inoculated with a five strain cocktail of *Salmonella* at 10^5 cfu/ml. Populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. were higher for fresh-cut cantaloupe than for fresh-cut watermelon and honeydew immediately after preparation. Populations of *Salmonella*, aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* ssp. in fresh-cut melons left at room temperature for up to 5 h before refrigeration were significantly ($P<0.05$) higher than populations in fresh-cut melons stored at 5 °C immediately after preparation. Populations of *Salmonella* recovered in fresh-cut melon after inoculation with the cocktail of *Salmonella* strains averaged 2 $\log_{10}$ cfu/g for all three types of melons. Populations in fresh-cut watermelon and honeydew pieces declined by 1 log when stored immediately at 5 °C for 12 days, while the populations in fresh-cut cantaloupe did not show significant ($P>0.05$) changes. Populations of *Salmonella* in fresh-cut melons stored immediately at 10 °C for 12 days increased significantly ($P<0.05$) from 2.0 to 3.0 $\log_{10}$ cfu/g in watermelon, 1.9 to 3.0 $\log_{10}$ cfu/g in honeydew and 2.0 to 3.6 $\log_{10}$ cfu/g in cantaloupe pieces. Holding freshly prepared, contaminated fresh-cut melon pieces at 22 °C for 3 h or more prior to refrigerated storage would increase the chances of *Salmonella* proliferation, especially if the fresh-cut melons were subsequently stored at an abusive temperature.
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Keywords: Storage temperature; Fresh-cut; Watermelon; Honeydew; Cantaloupe; *Salmonella*

1. Introduction

The microflora of foods is of practical significance to producers, processors and consumers. Fruits and vegetables are frequently in contact with soil, insects, animals, and humans during growth in the field and harvesting and may become contaminated with human pathogens. The level of sanitation during processing, shipping and at retail outlets as well as the initial microbiological load are of primary importance to the quality, shelf stability and safety of fresh and fresh-cut produce (Beuchat, 1995; Brackett, 1992; Cousin et al., 2001; Hurst and Schuler, 1992). Washing is one of the very first processing operations to which melons are subjected in fresh-cut processing; yet, their surfaces are not free from natural contaminants, and by the time they reach the packinghouse, most fresh produce including melons harbor populations of 10^4–10^6 micro-organisms/g (Beuchat, 1995; Brackett, 1992; Castillo et al., 2004).

Prepared fresh-cut melon in the supermarket is becoming very popular with the US consumer due to the benefits of a diet rich in fruits and vegetables. To date, fresh-cut melon is prepared in the supermarket or by regional distributors. National distribution is difficult due to quality and safety

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concerns. Five multi-state outbreaks of salmonellosis have been associated epidemiologically with cantaloupes. The first in 1990 involved *Salmonella* Chester which affected 245 individuals (two deaths) in 30 states (Mead et al., 1999; Rise et al., 1990). The second in 1991 involved more than 400 laboratory confirmed *Salmonella* Poona infections and occurred in 23 states and Canada (CDC, 1991). The most recent multi-state outbreaks (occurring in years 2000, 2001, and 2002) were due to *Salmonella* Poona (CDC, 2002). In all outbreaks noted so far, reports mentioned melons which had been precut and held at unknown temperatures for some period of time at retail prior to being purchased and consumed. The inner flesh of fresh-cut melons is composed mainly of parenchyma cells (Grigorieva et al., 1965) containing sugars, organic acids, and other substances that may support microbial growth as observed in our prior study (Ukuku and Sapers, 2001). Golden and Kautter (1993) reported growth of *Salmonella* spp. in cantaloupe, watermelon, and honeydew melons stored at 23 °C. Other investigators (Escartin et al., 1989) have reported that interior watermelon tissues support the growth of *Salmonella* spp. at 23 °C.

To control microbial growth in fresh-cut melons the Food and Drug Administration recommended keeping fresh-cut melons at 41°F or below in a refrigerated case and to date mark fresh-cut melons held for more than 24 h before storage at 41°F and should be discarded within 7 days (FDA, 2000). At home, after preparation fresh-cut melon may be left at room temperature for a number of hours before consumption or refrigeration of the leftovers for later use. In this study, our objective was to investigate the effect of time before storage and storage temperature on survival of *Salmonella* inoculated on fresh-cut watermelon, cantaloupe and honeydew. Knowledge of the level of microflora on whole and fresh-cut melon, the impact of time before refrigerated storage and the storage temperature on microbial populations should provide guidance to the food service industry and consumers as well as help fresh-cut processors in implementing HACCP plans and good manufacturing practices (GMPs).

2. Materials and methods

2.1. Bacterial strains, growth conditions, and inoculum preparation

Five *Salmonella* strains were used in this study: *Salmonella* Newport 02-216, sprout-related outbreak; *Salmonella* Poona 418, meat; *Salmonella* Hidalgo 02-517-2, cantaloupe; *Salmonella* Typhimurium 45, cantaloupe; *Salmonella* St. Paul FSIS 039, cantaloupe. Bacteria were maintained on brain–heart infusion agar (BHIA, BBL/Difco, Sparks, Maryland) slants held at 4 °C. Prior to use, each culture was subjected to two successive transfers by loop inocula to 5 ml brain–heart infusion broth (BHIB, BBL/Difco). A final transfer of 0.2 ml was made into 20 ml BHIB with incubation at 36 °C for 18 h under static conditions. Bacterial cells were harvested by centrifugation (10,000*g*, 10 min) at 4 °C, and the cell pellets were washed in salt–peptone [0.85% NaCl, 0.05% Bacto-peptone (BBL/Difco)]. The cell pellets were used to prepare the inoculum consisting of the individual bacterial strains at approximately 1.3×10^5 cfu/ml in 31 of 0.1% (w/v) peptone-water.

2.2. Washing treatments

Unwaxed whole cantaloupes (Western shippers), honeydew and watermelons purchased from a local produce distributor were allowed to come to room temperature (~22 °C) overnight before use. A total of 12 of each different melon (cantaloupes, honeydew and watermelons) were used for this study. In other to mimic fresh-cut preparation at home, individual melons were washed under running tap water (~19 °C) for 5 min. Washed melons were placed on crystallizing dishes inside a biosafety cabinet to dry for 1 h and then treated as stated below.

2.3. Preparation of fresh-cut pieces and inoculation with Salmonella

To prepare fresh-cut pieces, whole cantaloupe, watermelon and honeydew melons were cut into four sections using a sterile knife. Prior to use cutting boards and knives were sanitized in 200 ppm chlorine solution prepared by diluting Clorox® commercial bleach containing 5.25% NaOCl in sterile tap water and adjusting the pH to 6.4 ± 0.1 by adding citric acid (Mallinckrodt, Paris, Kentucky). The rinds were removed, and the flesh was cut into ~3 cm cubes. Fresh-cut pieces from each melon type (800 g) and a mixture of fresh-cut pieces from the three melon types were submerged in a cocktail of *Salmonella* inoculum at 6.6×10^5 cfu/ml for 30 s. Inoculated fresh-cut pieces were placed on a perforated dish for 1 h inside a biosafety cabinet to drain any excess fluid. For storage experiments, 250 g samples of each inoculated fresh-cut watermelon, honeydew, cantaloupe and the mixed fresh-cut pieces were placed inside 9.75 inch diameter three pocket plastic bowls (Mach #2, Rock-Tenn Company, Franklin Park, Illinois, USA) and stored at 5, 10 or 22 °C for 12–15 days. A second batch of samples immediately stored at 5 °C for 3 h was pulled out of the refrigerator and microbial determination performed within 1 h. This was done to investigate the behavior of *Salmonella* in contaminated fresh-cut melons during short time storage at 5 °C before consumption. The third batch of samples was left at room temperature for up to 5 h before microbial determination. This particular study was designed to investigate the behavior of *Salmonella* in contaminated fresh-cut melons left at room temperature for up to 5 h before consumption. Samples from all batches were subjected to microbiological analysis on day 0 (within 5 h of being prepared) and periodically for up to 12–15 days during storage at 5, 10 or 22 °C.

2.4. Enumeration of native microflora on whole melon surfaces

Cantaloupe, watermelon and honeydew surfaces were randomly cut with a sterilized stainless steel cork-borer to produce rind plugs of 22 mm in diameter with an external rind surface area (πr^2) of 3.80 cm^2. A total of about 100 rind plugs per melon were obtained. The interior flesh adhering to the rind plugs was trimmed off using a sterilized stainless steel knife. Melon rind plugs (70, ~25 g) were blended (Waring commercial blender, speed set at level 5 for 1 min) with 75 ml of 0.1% PW. Decimal dilutions of the samples were made with 0.1% PW and 0.1 ml plated in duplicate onto a range of media. Plate Count Agar (PCA, BBL/ Difco Becton Dickinson Sparks, Maryland, USA), with incubation at 30 °C for 3 days, was used for enumeration of mesophilic aerobes (Messer et al., 1984). *Pseudomonas* spp. were enumerated on *Pseudomonas* isolation agar (BBL/Difco) with incubation at 27 °C for 3 days. Yeast and mold were enumerated with incubation at 25 °C for 5 days according to Norris and Ribbons (1971) using Czapek Malt Agar (CMA, Sigma, St. Louis, Missouri, USA).

2.5. Microbiological analysis of fresh-cut pieces

Populations of mesophilic aerobic bacteria, yeast and mold, and *Pseudomonas* spp. and inoculated *Salmonella* in fresh-cut pieces were determined. Fresh-cut pieces (100 g) and 200 ml of 0.1% peptone-water were added to a Stomacher® bag and pummeled for 30 s in a Stomacher® model 400 (Dynatech Laboratories, Alexandria, Virginia, USA) at medium speed. Aliquots (0.1 ml) of undiluted homogenate and decimal serial dilutions, prepared in 0.1% peptone-water, were plated in duplicate onto a range of media. Aerobic mesophilic bacteria, *Pseudomonas* spp. and yeast and mold were enumerated as stated above. *Salmonella* was enumerated by plating 0.1 ml samples on XLT4 agar (BBL/Difco, Sparks, Maryland, USA) with incubation at 35 °C for 48 h. For comparison, a pure culture of *Salmonella* was plated on XLT4 agar, incubated as above, and run parallel with the samples. Selected black or black-centered colonies from the agar plates were confirmed to be *Salmonella* according to the FDA Bacteriological Analytical Manual following conventional biochemical methods (Andrews et al., 1995) as well as serological assays using latex agglutination (Oxoid, Ogdensburg, New York, USA).

2.6. Data analysis

All experiments were done in triplicate with duplicate samples being analysed at each sampling time. Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System Program (SAS Institute, Cary, North Carolina, USA). Significant differences ($P<0.05$) between mean values of number of cells at specific waiting time before storage were determined by the Bonferroni LSD method (Miller, 1981).

3. Results and discussion

The populations of mesophilic aerobes, yeast and mold and *Pseudomonas* spp. determined on whole cantaloupe, watermelon and honeydew surfaces and the numbers transferred to fresh-cut pieces are shown in Table 1. Populations of mesophilic aerobes determined on whole cantaloupe surfaces were significantly ($P<0.05$) higher by 2.5 and 3.8 $\log_{10}$ cfu/cm^2 than the populations on watermelon and honeydew, respectively (Table 1). Yeast and mold and *Pseudomonas* spp. populations on watermelon and honeydew surfaces were not significantly ($P>0.05$) different from each other, but were significantly lower (2 and 2.5 $\log_{10}$ cfu/cm^2, respectively) than the populations on cantaloupe. The data supports our previous report of higher microbial populations on whole cantaloupe surfaces than on whole honeydew melon surfaces (Ukuku et al., 2004). In this study, microbial populations determined on the surface of water melon were also significantly ($P<0.05$) lower than for cantaloupe. Also, lower microbial populations were determined in fresh-cut honeydew and watermelon than for fresh-cut cantaloupe (Table 1).

Table 1
Populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. on cantaloupe, watermelon and honeydew surfaces

Microflora	Population (Log_{10} cfu)[a]					
	Cantaloupe		Watermelon		Honeydew	
	Whole (cfu/cm^2)	Fresh-cut (cfu/g)	Whole (cfu/cm^2)	Fresh-cut (cfu/g)	Whole (cfu/cm^2)	Fresh-cut (cfu/g)
Aerobic mesophilic bacteria	6.6±0.15 A	3.2±0.11 A	4.1±0.13 B	0.8±0.02 B	2.8±0.13 C	0.9±0.02 B
Yeast and mold	2.8±0.12 A	0.6±0.05 A	0.8±0.05 B	BD[b]	0.8±0.04 B	BD
Pseudomonas spp.	2.9±0.06 A	0.8±0.07 A	0.4±0.02 B	BD	0.3±0.06 B	BD

[a]Values represent means±SD for data from three experiments with duplicate determinations per experiment. Means for each class of organism in the same row for whole or fresh-cut melon not followed by the same letter are significantly ($P<0.05$) different.
[b]BD = below limit of detection (1 cfu/g).

3.1. Effect of holding period before refrigeration on native microflora of fresh-cut melons

Populations of native microflora transferred from whole melon surfaces to fresh-cut pieces during fresh-cut preparation and the populations attained after fresh-cut pieces were left at room temperature for up to 5 h are shown in Fig. 1. The populations of native microflora transferred from melon surfaces to fresh-cut pieces are dependent on the type of melon. Fresh-cut cantaloupes and mixed fresh-cut pieces had larger microbial populations than did honeydew and watermelon pieces. Spoilage organisms (yeast and mold) transferred to fresh-cut pieces were below the detection limit (<1 cfu/g).

The population of mesophilic aerobic bacteria on all fresh-cut melons left at room temperature (22 °C) for up to 5 h before refrigeration at 5 °C for 3 h increased approximately by 1 $\log_{10}$ cfu/g for all types of fresh-cut melons (from an average of 0.8 ± 0.02 to 1.6 ± 0.12 $\log_{10}$ cfu/g in watermelon, 0.9 ± 0.02 to 1.8 ± 0.10 $\log_{10}$ cfu/g in honeydew, 3.2 ± 0.11 to 3.9 ± 0.16 $\log_{10}$ cfu/g in cantaloupe and 3.8 ± 0.14 to 4.7 ± 0.17 $\log_{10}$ cfu/g in mixed-fresh-cut melons) (Fig. 1). Populations of yeast and mold in fresh-cut cantaloupe and mixed fresh-cut melons left at room temperature (22 °C) for 5 h before refrigeration (5 °C) for 3 h increased slightly from 0.6 to 1.3 and 0.9 to 1.7 cfu/g, respectively. Yeast and mold populations in all fresh-cut honeydew and watermelon samples were below detection up to 2 h. Populations of *Pseudomonas* spp. in all fresh-cut melon increased by approximately 1 log during storage for 5 h at 22 °C, similar to that for aerobic mesophilic bacteria. The populations of mesophilic aerobic bacteria, yeast and molds and *Pseudomonas* spp. on fresh-cut pieces stored at 5 °C for 3 h immediately after preparation did not show significant ($P>0.05$) changes and were comparable to the populations determined immediately after fresh-cut preparation (data not shown).

3.2. Effect of immediate refrigeration on microbial quality during long-term storage

Populations of native microflora on fresh-cut melons stored at 5 °C for up to 12 days immediately after processing are shown in Fig. 2. Populations of yeast and mold in fresh-cut watermelon and honeydew pieces were not detectable until day 8 at 5 °C storage. However, the organism was detected in fresh-cut cantaloupe pieces at day 2, and the populations reached approximately 1 log cfu/g at day 12. Aerobic mesophilic bacteria and *Pseudomonas* spp. were detected in all fresh-cut pieces beginning with the initial sampling, and the populations were highest in fresh-cut cantaloupe and mixed fruits. Visible evidence of microbial spoilage was not apparent in fresh-cut melon samples except cantaloupe pieces which had visible mold at day 10 and above. Watermelon pieces had similar microbial populations at each sampling time as in honeydew but in contrast to honeydew were soggy or translucent at day 4.

3.3. Effect of holding period before refrigeration of fresh-cut melon on Salmonella population

Initial populations of *Salmonella* recovered in all fresh-cut pieces immediately after inoculation averaged 2 log cfu/g (Fig. 3 and Table 2). There were no significant increases in *Salmonella* population on fresh-cut pieces held at 22 °C for up to 2 h. Above 3 h, the populations were significantly ($P<0.05$) higher for all but watermelon with the highest

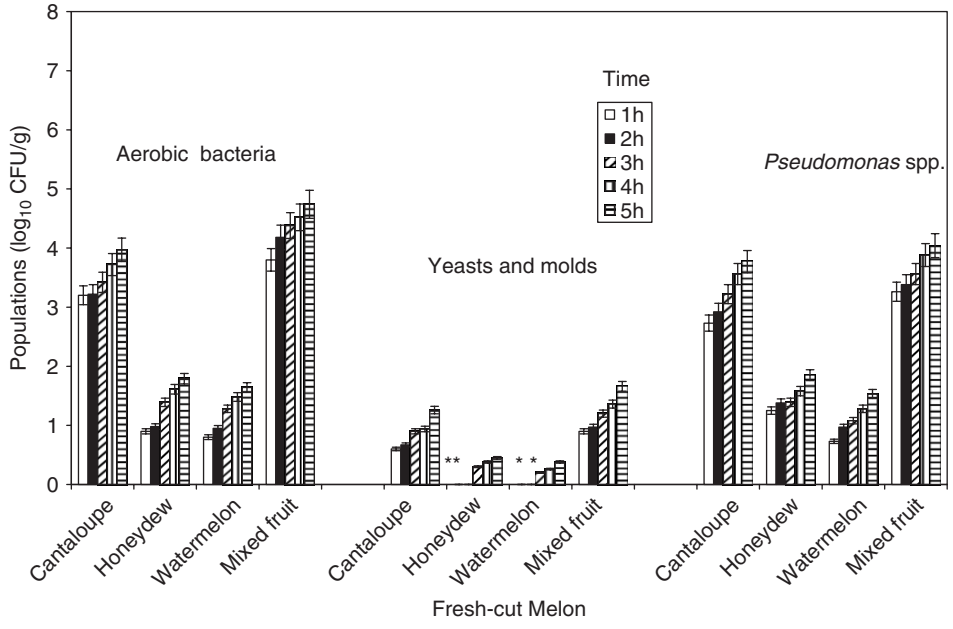

Fig. 1. Populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. on fresh-cut melon left at 22 °C for up to 5 h before refrigeration at 5 °C for 3 h. Values are mean ± SD for three experiments with duplicate determinations. *Below detection (<1 cfu/g).

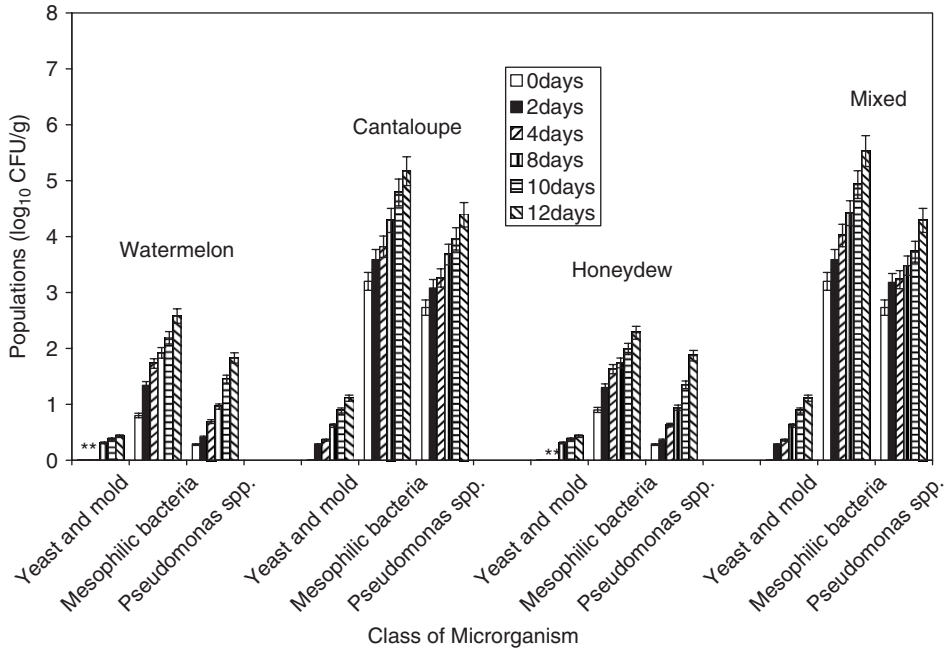

Fig. 2. Populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. on fresh-cut melons during storage at 5 °C for 12 days. Values are mean ± SD for three experiments with duplicate determinations. *Below detection (< 1 cfu/g).

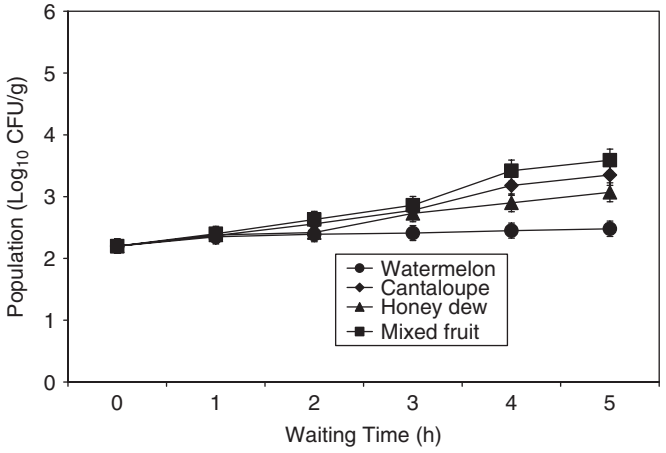

Fig. 3. Populations of *Salmonella* inoculated on fresh-cut melons left at 22 °C for up to 5 h before refrigeration at 5 °C for 5 h. Fresh-cut melons were dip-inoculated in 6.5×10^5 cfu/ml *Salmonella* suspensions for 30 s. Values are mean ± SD for three experiments with duplicate determinations.

populations determined in fresh-cut cantaloupe or the mixed fresh-cut pieces. Population of the pathogen in watermelon pieces did not change significantly ($P > 0.05$) during 5 h of waiting at 22 °C before refrigeration.

Salmonella populations in all fresh-cut melons stored at 5, 10 or 22 °C for up to 12 days are shown in Fig. 4. At day 0, *Salmonella* recovered from inoculated fresh-cut melons averaged approximately 2.0 $\log_{10}$ cfu/g for watermelon, 1.9 $\log_{10}$ cfu/g for honeydew pieces, and 2.2 $\log_{10}$ cfu/g for cantaloupe and for mixed fruits. With storage at 5 °C, the *Salmonella* population in fresh-cut watermelon and honeydew declined by 1 log over 10 days, while there was no significant decline in fresh-cut cantaloupe pieces and mixed fruits throughout storage. In fresh-cut melons stored at 10 °C, *Salmonella* populations increased significantly ($P < 0.05$) from 2.0 to 3.0 $\log_{10}$ cfu/g in watermelon, 1.9 to 3.0 $\log_{10}$ cfu/g in honeydew and 2.0 to 3.6 $\log_{10}$ cfu/g in cantaloupe pieces by day 12. Growth of this pathogen was more pronounced in cantaloupe and the mixed fresh-cut pieces than in honeydew or watermelon fresh-cut pieces (Fig. 4). In fresh-cut melons stored at 22 °C for 12 days, *Salmonella* populations increased significantly ($P < 0.05$) and were higher by 0.8 $\log_{10}$ cfu/g in watermelon, and by 3.0 $\log_{10}$ cfu/g in honeydew at day 12. Also, *Salmonella* populations in fresh-cut cantaloupes and mixed fruits increased and reached a plateau at day 2 before declining to a number lower than the initial starting populations.

Results of a second study designed to investigate the behavior of *Salmonella* in the presence of native microflora of mixed fresh-cut melons during storage at abusive temperatures (10 and 22 °C) are shown in Fig. 5. *Salmonella* populations increased by 2 log at day 6 and 2.5 log at day 3 during storage at 10 and 22 °C, respectively. Similarly, yeast and mold, mesophilic aerobic bacteria and *Pseudomonas* spp. grew in mixed fresh-cut melons stored at 10 and 22 °C. At 10 °C storage, the population of *Salmonella* started to decline at day 9 and by day 15 the total *Salmonella* population recovered from the mixed fruits averaged 2 log/g. In fresh-cut pieces stored at 22 °C, the population of *Salmonella* declined after day 3, and at day 9, the experiment was stopped due to extensive odor, presence of molds and high aerobic plate counts.

It is possible that yeast and mold was antagonistic to the survival of *Salmonella* in the fresh-cut pieces. In our previous study, we reported that addition of yeast and

Table 2
Population of *Salmonella* on fresh-cut melon stored immediately at 5 °C for 3 h or left at 22 °C for 3 h before storage at 5 °C

Fresh-cut melon	Population ($\log_{10}$ cfu/g)[a]			
	Stored at 5 °C immediately after preparation	Held at 22 °C for 3 h before storage at 5 °C	Held at 22 °C for 5 h before storage at 5 °C	Held at 5 °C for 3 h, after preparation
Watermelon	2.1 ± 0.09^{B}	2.0 ± 0.04^{C}	2.2 ± 0.10^{C}	1.6 ± 0.09^{B}
Honeydew	1.9 ± 0.04^{C}	2.0 ± 0.10^{C}	2.6 ± 0.10^{B}	1.5 ± 0.06^{B}
Cantaloupe	2.2 ± 0.10^{B}	2.5 ± 0.11^{B}	3.5 ± 0.15^{A}	1.9 ± 0.11^{A}
Mixed melons*	2.5 ± 0.10^{A}	2.8 ± 0.12^{A}	3.6 ± 0.13^{A}	2.0 ± 0.10^{A}

Means in the same column for each fresh-cut melon per treatment not followed by the same letter are significantly ($P < 0.05$) different.
[a]Values represent means ± SD for data from three experiments with duplicate determinations per experiment.

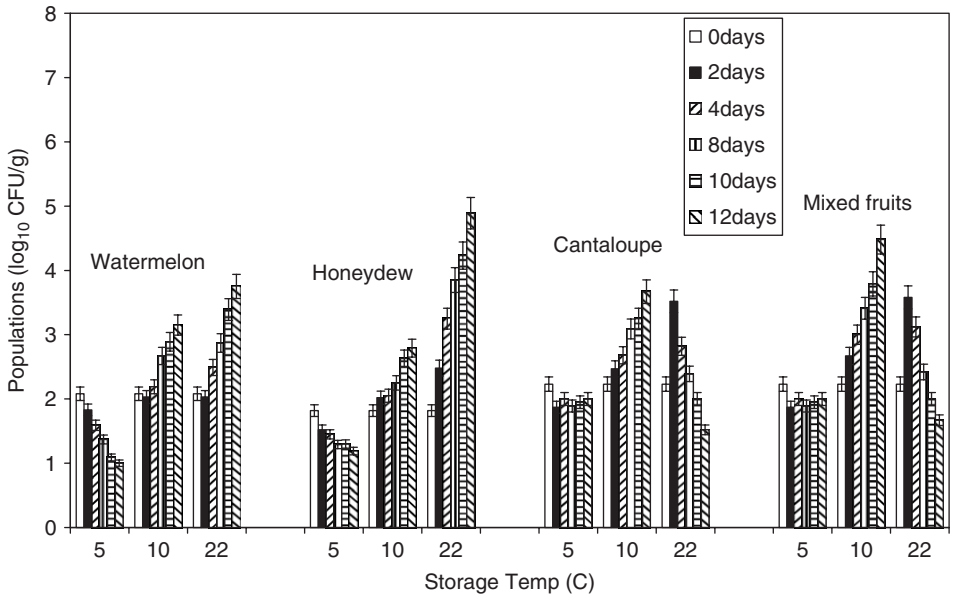

Fig. 4. Survival of *Salmonella* in the presence of indigenous microflora on fresh-cut melon stored at 5, 10 or 22 °C for 12 days. Fresh-cut melons were dip-inoculated in 6.5×10^5 cfu/ml *Salmonella* suspensions for 30 s. Values are mean ± SD for three experiments with duplicate determinations.

mold to sterile cantaloupe rind homogenates was highly inhibitory to growth and survival of the *L. monocytogenes*. Also, we reported higher populations of *L. monocytogenes* grown in sterile cantaloupe tissue homogenates than in non-sterile homogenates (Ukuku et al., 2004). Although microbial growth in fresh-cut melons due to holding at 22 °C for 3 h after preparation and prior to refrigeration was not significant, even the small increase in population of *Salmonella* (Fig. 3) suggests the need for immediate refrigeration after fresh-cut preparation.

The visual symptoms of deterioration of fresh-cut produce are flaccidity due to loss of water, changes in color resulting from oxidative browning at the cut surfaces, and microbial contamination (King and Bolin, 1989; Varoquaux and Wiley, 1994; Brecht, 1995). In this study, during storage at 10 °C, fresh-cut cantaloupe pieces were the first to show presence of mold growth as early as 4 days, followed by honeydew at day 6, with no appearance of mold on watermelon pieces over the 12-day storage period. Fresh-cut melon held at 22 °C for up to 6 days looked slimy with higher yeast and mold counts, but had lower *Salmonella* counts than fresh-cut pieces stored at 10 °C (Fig. 5).

The results of previous studies designed to determine the shelf-life of minimally processed honeydew and cantaloupe melon, kiwifruit, papaya, and pineapple stored at 4 °C indicated that both the length of shelf-life and type of spoilage were related to the type of fruit (O'Connor-Shaw et al., 1994; Nguyen–The and Carlin, 1994). The authors suggested that the microflora of fruit pieces need to be studied to set appropriate criteria for quality assessment. In this study, we determined three categories of microbes (aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp.) on the surfaces of whole cantaloupe, honeydew melon and watermelon. The populations of all three categories of micro-organisms were found to be higher on cantaloupe than on honeydew melon or watermelon. The higher populations of the native microflora on cantaloupe

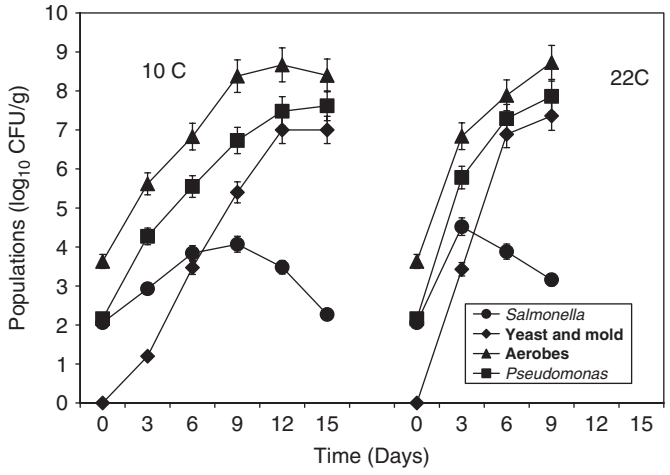

Fig. 5. Survival of *Salmonella* in the presence of indigenous microflora on fresh-cut mixed melon stored at 10 or 22 °C for up to 15 days. Fresh-cut melons were dip-inoculated in 6.5×10^5 cfu/ml *Salmonella* suspensions for 30 s. Values are mean ± SD for three experiments with duplicate determinations. Experiment stopped at 12 days of storage at 22 °C due to strong odor and spoilage.

rind than the honeydew and watermelon is most likely due to the rough surface of the cantaloupe rind compared to the relatively smooth surfaces of honeydew and watermelon. The extensive raised netting on the surface of cantaloupe melon no doubt provides more microbial attachments sites and helps to protect attached microbes from being washed from the surface, and possibly from environmental stresses such as UV radiation and desiccation (Ukuku and Fett, 2002; Ukuku, 2004). Surface irregularities such as roughness, crevices, and pits have been shown to increase bacterial adherence and reduce the ability of washing treatments to remove bacterial cells (ICMS, 1980). Micro-organisms embedded in plant tissues will be protected from chemicals such as chlorine that have little penetrating power during minimal processing of fruits and vegetables (Seo and Frank, 1999). Subsequent transfer of such protected micro-organisms including bacterial pathogens to the internal tissue during cutting has been reported in studies with cantaloupe (Ukuku and Sapers, 2001) and tomato (Lin and Wei, 1997). Transfer of microbial populations from melon surfaces to fresh-cut pieces has been reported (Ukuku and Sapers, 2001; Ukuku and Fett, 2002; Lin and Wei, 1997). In this study, the populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. determined in fresh-cut melon, which presumably were transferred during cutting, were higher in cantaloupes than in honeydew or watermelon pieces. Yeast and mold were suppressed in fresh-cut pieces stored at 5 °C. At 10 °C storage, all classes of the native microflora increased significantly, especially in cantaloupe pieces and mixed fresh-cut fruits (Fig. 5).

All melon-related foodborne outbreaks reported so far involved melons that were precut and held for some period of time prior to being purchased and consumed. Tamplin (1997) recommended that attention be directed to cleaning the melons at the time of cutting, using clean and sanitized utensils and surfaces to minimize contamination of the edible portion, and immediately consuming, or holding cut melon pieces at cold temperatures. The effect of holding times before refrigeration and also storage at refrigeration and abuse temperatures has been investigated (FDA, 2000). In this report, the FDA recommended keeping the cut melons at 41 °F or below in a refrigerated case (not just on top of ice), and that cut melons should be displayed for a maximum of 4 h without refrigeration, and then discarded. The result of our study (Fig. 3) is in agreement with the FDA recommendation. *Salmonella miami* and *Salmonella bareilly* were responsible for two salmonellosis outbreaks associated with precut wrapped watermelon (Gayler et al., 1955). The investigators showed that the interior watermelon tissue could become contaminated if *Salmonella* was present either on the rind of the watermelon or on the knife used for slicing. However, they failed to report the initial inoculum size or the final population attained on the watermelon flesh. Transfer of *Salmonella* from the surface of tomatoes to the interior during cutting has been reported (Lin and Wei, 1997). Similarly, Ukuku and Sapers (2001) reported transfer of *Salmonella* from cantaloupe surfaces to fresh-cut pieces which could be reduced to below the detectable limit when the inoculum size was reduced by a sanitizer treatment. Golden and Kautter (1993) reported a 5 log cfu/g increase of *Salmonella* spp. on fresh-cut cantaloupe, honeydew and watermelon from an initial population of 2 log cfu/g after incubation at 23 °C for 24 h. Other investigators reported lower than 5 log cfu/g increase of *Salmonella* on watermelon in distilled water (20% wt/vol) (Escartin et al., 1994; Del Rosario and Beuchat, 1995).

Populations of *Salmonella* spp. on fresh-cut watermelon and honeydew surfaces slightly decreased while the populations on cantaloupe and mixed fruits remain the same during storage at 5 °C for 12 days (Fig. 4). When fresh-cut watermelon, honeydew, cantaloupe and the mixed fresh fruits inoculated with *Salmonella* spp. were stored at 10 and 22 °C the pathogen were significantly ($P < 0.05$) greater than on the fresh-cut melons stored at 5 °C. In this study, a 1–1.5 log cfu/g increase of *Salmonella* spp. on fresh-cut watermelon and honeydew was observed after storage at 10 °C for 12 days. However, the population of the pathogen in fresh-cut piece stored at 22 °C for 12 days increased by 2–3 log cfu/g in watermelon and cantaloupe, respectively. The population in cantaloupe pieces and the mixed fruits increased by only 2 log cfu/g. The 2 log cfu/g increase of *Salmonella* spp. on fresh-cut cantaloupe and the mixed fruits may be attributed to the yeast and mold counts which were higher, and may have antagonized the survival and growth of the pathogen (Fig. 5).

In conclusion, the populations of aerobic mesophilic bacteria, yeast and mold and *Pseudomonas* spp. recovered from fresh-cut cantaloupes were higher than those determined in honeydew and watermelon pieces. The *Salmonella*

population in inoculated fresh-cut melon pieces declined slightly throughout 12 days storage at 5 °C but increased during storage at 10 °C. Holding freshly prepared, contaminated fresh-cut melon pieces at 22 °C for three or more hours prior to refrigerated storage would increase the chances of *Salmonella* proliferation, especially if the fresh-cut melons were subsequently stored at an abusive temperature.

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